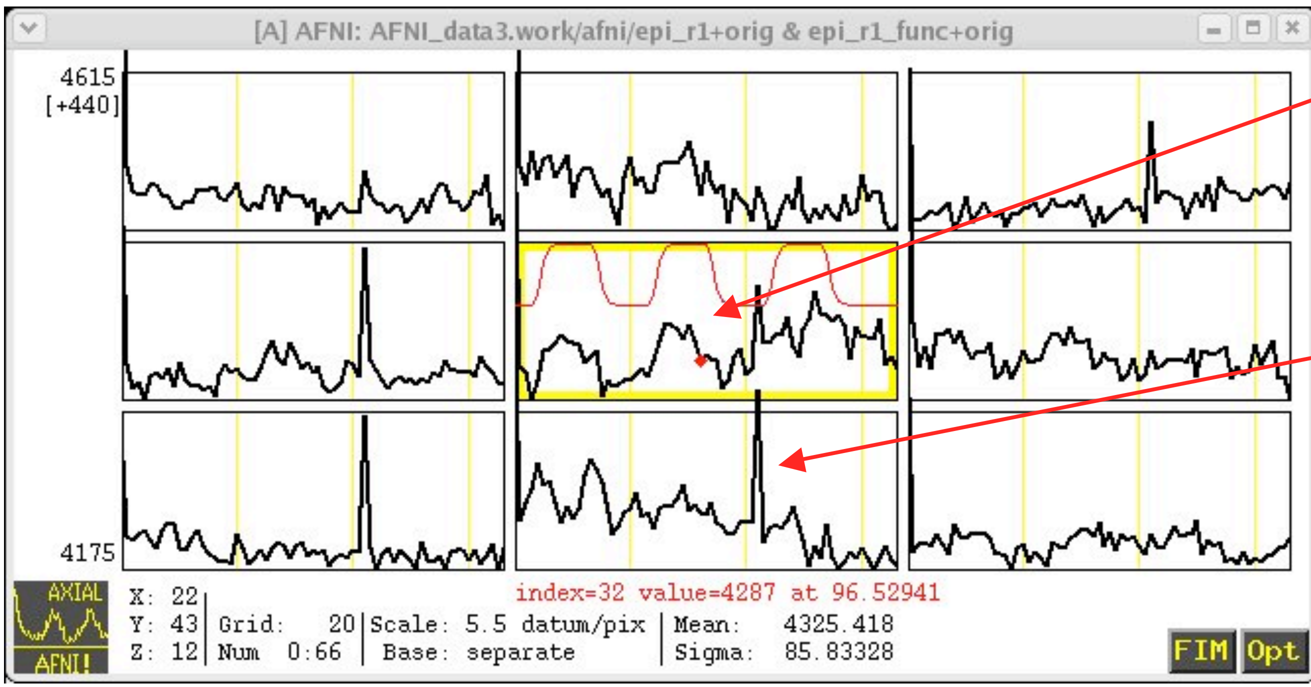
Sample Data Analysis: Simple Regression

- Enough theory (for now: more to come later!)
- To look at the data: type `cd AFNI_data3/afni` ; then `afni`
- `Switch Underlay` to dataset `epi_r1`
  - Then `Axial Image` and `Graph`
  - `FIM→Pick Ideal` ; then click `afni/epi_r1_ideal.1D` ; then `Set`
  - Right-click in image, `Jump to (ijk)`, then `22 43 12`, then `Set`



- Data clearly has activity in sync with reference
  - 30 s blocks
- Data also has a big spike, which is very annoying
  - Subject head movement!

Preparing Data for Analysis

- Six preparatory steps are common:
  - Temporal alignment: program **3dtshift**
  - Image registration (AKA realignment): program **3dvolreg**
  - Image smoothing: program **3dmerge**
  - Image masking: program **3dClipLevel** or **3dAutomask**
  - Conversion to percentile: programs **3dTstat** and **3dcalc**
  - Censoring out time points that are bad: program **3dToutcount** or **3dTqual**

- Not all steps are necessary or desirable in any given case
- In this first example, will only do registration, since the data obviously needs this correction

Data Analysis Script

- **In file epi_r1_regress:**

```
3dvolreg -base 3                 \
         -verb                   \
         -prefix epi_r1_reg      \
         -1Dfile epi_r1_mot.1D   \
         epi_r1+orig
```

- 3dvolreg (3D image registration) will be covered in detail in a later presentation
- filename to get estimated motion parameters

- 3dDeconvolve = regression code

```
3dDeconvolve                            \
    -input epi_r1_reg+orig              \
    -nfirst     3                       \
    -num_stimts 1                       \
    -stim_times 1 epi_r1_times.1D       \
                 'BLOCK(30)'            \
    -stim_label 1 AllStim               \
    -tout                               \
    -bucket epi_r1_func                 \
    -fitts   epi_r1_fitts               \
    -xjpeg epi_r1_Xmat.jpg              \
    -x1D epi_r1_Xmat.x1D
```

- Name of <u>input</u> dataset (from 3dvolreg)
- Index of first sub-brick to process [skipping #0-2]
- Number of input model time series
- Name of <u>input</u> stimulus class timing file (τ's)
- and type of HRF model to fit
- Name for results in AFNI menus
- Indicates to output t-statistic for β weights
- Name of output "bucket" dataset (statistics)
- Name of output model fit dataset
- Name of image file to store **X** [AKA **R**] matrix
- Name of text file in which to store **X** matrix

- Type **tcsh epi_r1_regress** ; then wait for programs to run

Text Output of the `epi_r1_decon` script

* `3dvolreg` output

```
++ 3dvolreg: AFNI version=AFNI_2007_05_29_1644 (Sep  5 2007) [64-bit]
++ Reading input dataset ./epi_r1+orig.BRIK
++ Edging: x=3 y=3 z=2
++ Initializing alignment base
++ Starting final pass on 67 sub-bricks: 0..1..2..3.. *** ..63..64..65..66..
++ CPU time for realignment=5.35 s  [=0.0799 s/sub-brick]
++ Min : roll=-0.103  pitch=-1.594  yaw=-0.038  dS=-0.354  dL=-0.021  dP=-0.191
++ Mean: roll=-0.047  pitch=+0.061  yaw=+0.023  dS=+0.006  dL=+0.032  dP=-0.076
++ Max : roll=+0.065  pitch=+0.290  yaw=+0.055  dS=+0.050  dL=+0.120  dP=+0.113
++ Max displacement in automask = 2.46 (mm) at sub-brick 42 } Maximum movement estimate
++ Wrote dataset to disk in ./epi_r1_reg+orig.BRIK
```

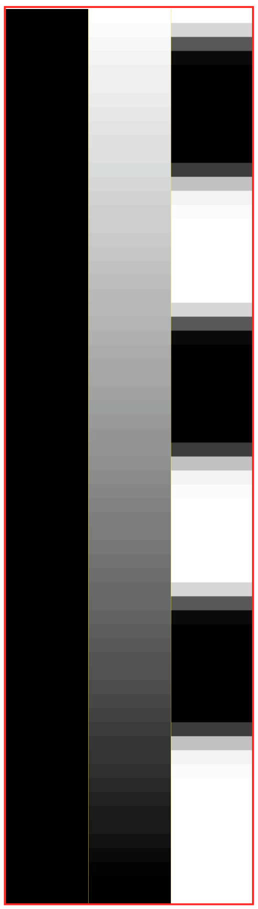
* `3dDeconvolve` output

```
++3dDeconvolve: AFNI version=AFNI_2007_05_29_1644 (Sep  5 2007) [64-bit]
++ Authored by: B. Douglas Ward, et al.
++ loading dataset epi_r1_reg+orig
*+ WARNING: Input polort=1; Longest run=201.0 s; Recommended minimum polort=2} Consider '-polort 2'
++ -stim_times using TR=3 seconds
++ '-stim_times 1' using LOCAL times
++ Wrote matrix image to file epi_r1_Xmat.jpg    } Output file indicators
++ Wrote matrix values to file epi_r1_Xmat.x1D   }
++ Signal+Baseline matrix condition [X] (64x3):  2.59165  ++ VERY GOOD ++   }
++ Signal-only matrix condition [X] (64x1):  1  ++ VERY GOOD ++             }
++ Baseline-only matrix condition [X] (64x2):  1.08449  ++ VERY GOOD ++     } Matrix Quality Assurance
++ -polort-only matrix condition [X] (64x2):  1.08449  ++ VERY GOOD ++      }
++ Matrix inverse average error = 5.62791e-16  ++ VERY GOOD ++              }
++ Calculations starting; elapsed time=0.238
++ voxel loop:0123456789.0123456789.0123456789.0123456789.0123456789. } Progress meter / pacifier
++ Calculations finished; elapsed time=1.417
++ Wrote bucket dataset into ./epi_r1_func+orig.BRIK     } Output file indicators
++ Wrote 3D+time dataset into ./epi_r1_fitts+orig.BRIK   }
++ #Flops=3.11955e+08  Average Dot Product=4.50251
```

* If a program crashes, we'll need to see this text output (at the very least)!

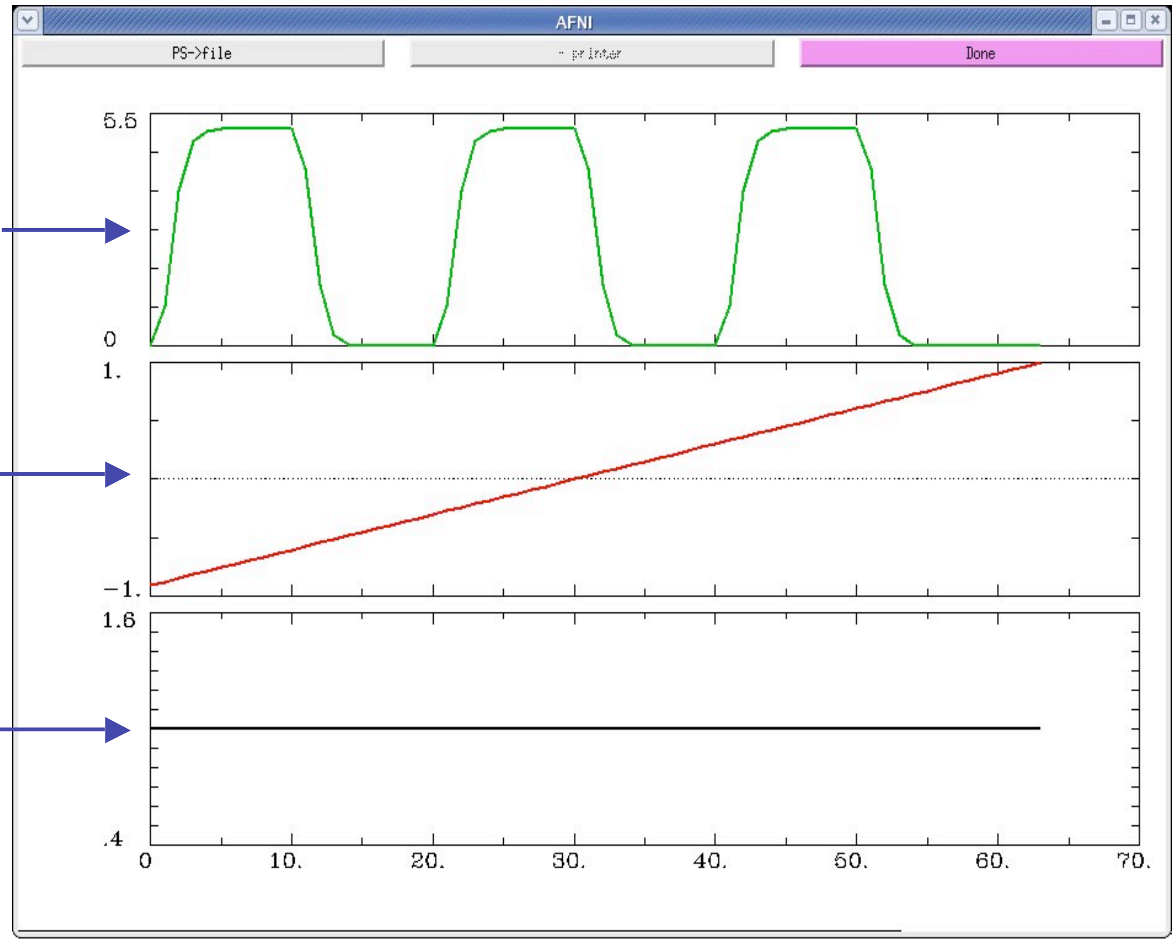
Stimulus Timing: Input and Visualization

epi_r1_times.1D = `9.0   69.0   129.0`
= times of start of each `BLOCK(20)`



X matrix columns

- HRF⊗timing
- Linear in ***t***
- All ones



`epi_r1_Xmat.jpg`

`1dplot -sepscl epi_r1_Xmat.x1D`

Look at the Activation Map

- Run **afni** to view what we've got (note: a subtle test over only 1 run)
  - **Switch Underlay** to `epi_r1_reg` (output from **3dvolreg**)
  - **Switch Overlay** to `epi_r1_func` (output from **3dDeconvolve**)
  - **Sagittal Image** and **Graph** viewers
  - **FIM→Ignore→3** to have graph viewer not plot 1st 3 time pts
  - **FIM→Pick Ideal** ; pick `epi_r1_ideal.1D` (output from **waver**)
- **Define Overlay** to set up functional coloring
  - **Olay→Allstim[0] Coef** (sets coloring to be from model fit β)
  - **Thr→Allstim[0] t-s** (sets threshold to be model fit t-statistic)
  - **See Overlay** (otherwise won't see the function!)
  - Play with threshold slider to get a meaningful activation map (e.g., $t=3$ is a decent threshold — more on thresholds later)
  - Again, use **Jump to (i j k)** to jump to index coordinates 22 43 12

More Looking at the Results

- Graph viewer: **Opt→Tran 1D→Dataset #N** to plot the model fit dataset output by **3dDeconvolve**
  - Will open the control panel for the **Dataset #N** plugin
  - Click first **Input** on ; then choose **Dataset epi_r1_fitts+orig**
  - Also choose **Color dk-blue** to get a pleasing plot
  - Then click on **Set+Close** (to close the plugin's control panel)
  - Should now see fitted time series in the graph viewer instead of data time series
  - Graph viewer: click **Opt→Double Plot→Overlay** on to make the fitted time series appear as an overlay curve
  - This tool lets you visualize the quality of the data fit
- Can also now overlay function on MP-RAGE anatomical by using **Switch Underlay** to **anat+orig** dataset
  - Probably won't want to graph the **anat+orig** dataset!

Setting the Threshold: Principles

- Bad things (i.e., errors):
  - False positives — activations reported that aren't really there ≡ **Type I errors** (i.e., activations from noise-only data)
  - False negatives — non-activations reported where there should be true activations found ≡ **Type II errors**
- Usual approach in statistical testing is to control the probability of a type I error (the "*p*-value")
- In FMRI, we are making <u>many</u> statistical tests: one per voxel (≈20,000+) — the result of which is an "activation map":
  - Voxels are colorized if they survive the statistical thresholding process

Start of Important Aside

Setting the Threshold: Bonferroni

- If we set the threshold so there is a 1% chance that any given voxel is declared “active” even if its data is pure noise (FMRI jargon: “uncorrected” *p*-value is 0.01):
    - And assume each voxel’s noise is independent of its neighbors (not really true)
    - With 20,000 voxels to threshold, would expect to get 200 false positives — this may be as many as the true activations! Situation: **Not so good**.
- Bonferroni solution: set threshold (e.g., on *t*-statistic) so high that uncorrected *p*-value is 0.05/20000=2.5e-6
    - Then have only a 5% chance that even a single false positive voxel will be reported
    - **Objection**: will likely lose weak areas of activation

Important Aside

Setting the Threshold: Spatial Clustering

- Cluster-based detection lets us lower the statistical threshold and still control the false positive rate
- **Two** thresholds:
  - **First**: a per-voxel threshold that is somewhat low (so by itself leads to a lot of false positives, scattered around)
  - **Second**: form clusters of spatially contiguous (neighboring) voxels that survive the first threshold, and keep only those clusters above a volume threshold — e.g., we don't keep isolated "active" voxels
- Usually: choose volume threshold, then calculate voxel-wise statistic threshold to get the overall "corrected" *p*-value you want (typically, corrected p=0.05)
  - No easy formulas for this type of dual thresholding, so must use simulation: AFNI program **`AlphaSim`**

Important Aside

AlphaSim: Clustering Thresholds

- Simulated for brain mask of 18,465 voxels
- Look for smallest cluster with corrected $p < 0.05$

Corresponds to sample data

Uncorrected *p*-value (per voxel)	Cluster Size / Corrected *p* (uncorrelated)	Cluster Size / Corrected *p* (correlated 5 mm)
0.0002	2 / 0.001	3 / 0.004
0.0004	2 / 0.008	4 / 0.012
0.0007	2 / 0.026	3 / 0.031
0.0010	3 / 0.001	4 / 0.007
0.0020	3 / 0.003	4 / 0.032
0.0030	3 / 0.008	5 / 0.013
0.0040	3 / 0.018	5 / 0.029
0.0050	3 / 0.030	6 / 0.012
0.0060	4 / 0.003	6 / 0.023
0.0070	4 / 0.004	6 / 0.036
0.0080	4 / 0.006	7 / 0.016
0.0090	4 / 0.010	7 / 0.027
0.0100	4 / 0.015	7 / 0.042

Can make activation maps for display with cluster editing using `3dmerge` program or in AFNI GUI
(new: Sep 2006)

End of Important Aside

Multiple Stimulus Classes

- The experiment analyzed here in fact is more complicated
  - There are 9 related communication stimulus types in a 3x3 design of Category by Affect (stimuli are shown to subject as pictures)
    - **Telephone, Email** & **Face-to-face** = categories
    - **Negative, Positive** & **Neutral** = affects
      - ✓telephone stimuli: tneg, tpos, tneu
      - ✓email stimuli: eneg, epos, eneu
      - ✓face-to-face stimuli: fneg, fpos, fneu
  - Each stimulus type has 3 presentation blocks of 30 s duration
  - Scrambled pictures are shown between blocks
  - 9 imaging runs, 64 useful time points in each
    - Originally, 67 TRs per run, but skip first 3 for MRI signal to reach steady state
    - So 576 TRs of data, in total
  - Already registered and put together into dataset `rall_vr+orig`

Regression with Multiple Model Files

- Script file **rall_decon** does the job:
- Run this script by typing **tcsh rall_decon** (takes a few minutes)

```
3dDeconvolve -input rall_vr+orig                                          \
    -jobs 2                                                               \  try to use 2 CPUs
    -concat '1D: 0 64 128 192 256 320 384 448 512'                        \  run indices
    -num_stimts 15                                                        \
    -stim_times 1 '1D: 0 * | | | 120 | | | | | 60'      'BLOCK(30)' \  stimulus times
    -stim_times 2 '1D: * * | | 120 | | 0 | | | | 120' 'BLOCK(30)' \  '|' indicates new run
    -stim_times 3 '1D: * * | 120 | | 60 | | | | | 0'   'BLOCK(30)' \  response model
    -stim_times 4 '1D: 60 * | | | | | 120 | 0 | |'     'BLOCK(30)' \
    -stim_times 5 '1D: * * | 60 | | 0 | | | 120 | |'   'BLOCK(30)' \
    -stim_times 6 '1D: * * | | 0 | | 60 | | | 60 |'    'BLOCK(30)' \
    -stim_times 7 '1D: * * | 0 | | | 120 | | 60 | |'   'BLOCK(30)' \
    -stim_times 8 '1D: 120 * | | | | | 60 | | 0 |'     'BLOCK(30)' \
    -stim_times 9 '1D: * * | | 60 | | | 0 | | 120 |'   'BLOCK(30)' \
    -stim_label 1 tneg -stim_label 2 tpos -stim_label 3 tneu              \  stimulus label
    -stim_label 4 eneg -stim_label 5 epos -stim_label 6 eneu              \
    -stim_label 7 fneg -stim_label 8 fpos -stim_label 9 fneu              \
```

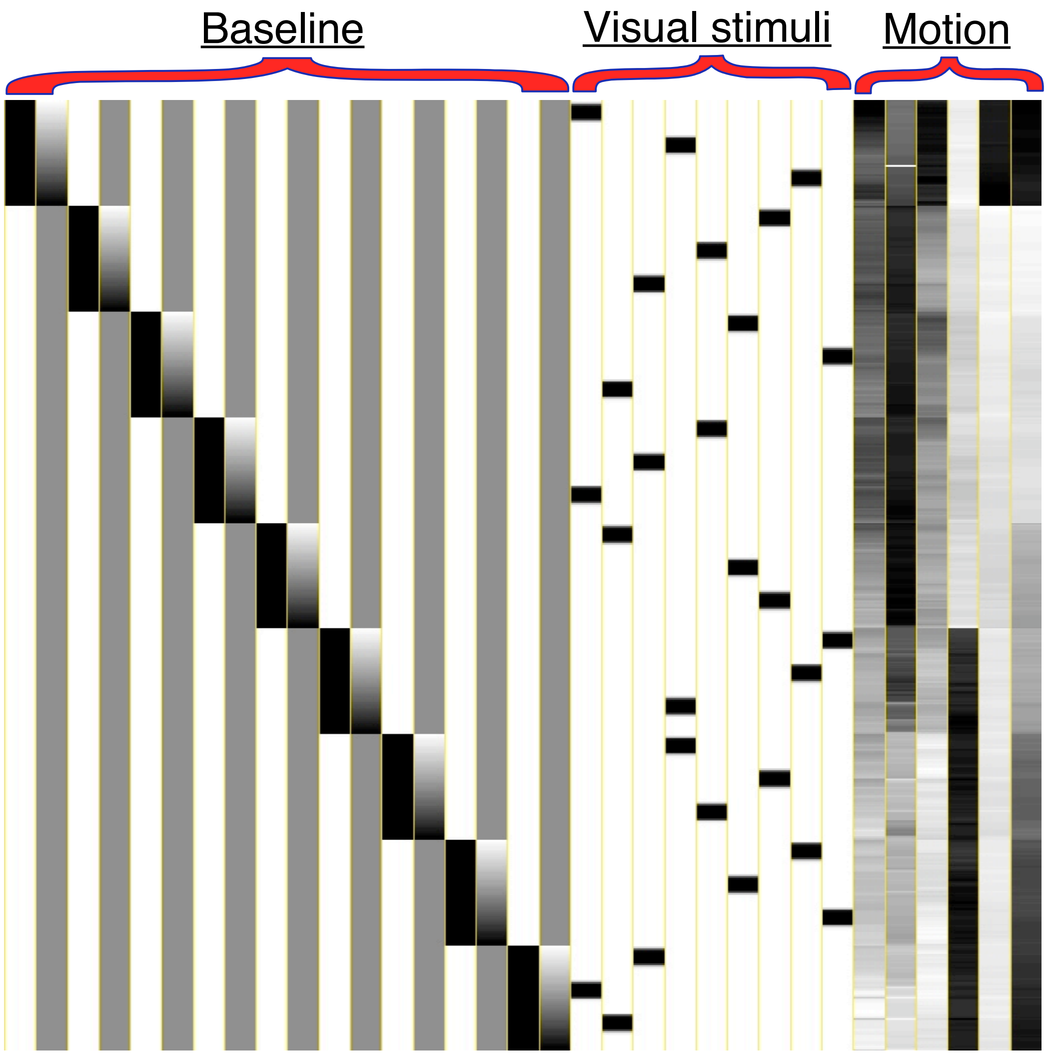
continued …

Regression with Multiple Model Files (continued)

```
-stim_file 10 motion.1D'[0]' -stim_base 10        \   motion regressor
-stim_file 11 motion.1D'[1]' -stim_base 11        \   apply to baseline
-stim_file 12 motion.1D'[2]' -stim_base 12        \
-stim_file 13 motion.1D'[3]' -stim_base 13        \
-stim_file 14 motion.1D'[4]' -stim_base 14        \
-stim_file 15 motion.1D'[5]' -stim_base 15        \
-gltsym 'SYM: tpos -epos' -glt_label 1 TPvsEP     \   symbolic GLT
-gltsym 'SYM: tpos -tneg' -glt_label 2 TPvsTNg    \   label the GLT
-gltsym 'SYM: tpos tneu tneg -epos -eneu -eneg'   \
        -glt_label 3 TvsE                         \
-fout -tout                                       \   statistic types to output
-bucket rall_func -fitts rall_fitts               \
-xjpeg rall_xmat.jpg -x1D rall_xmat.x1D
```

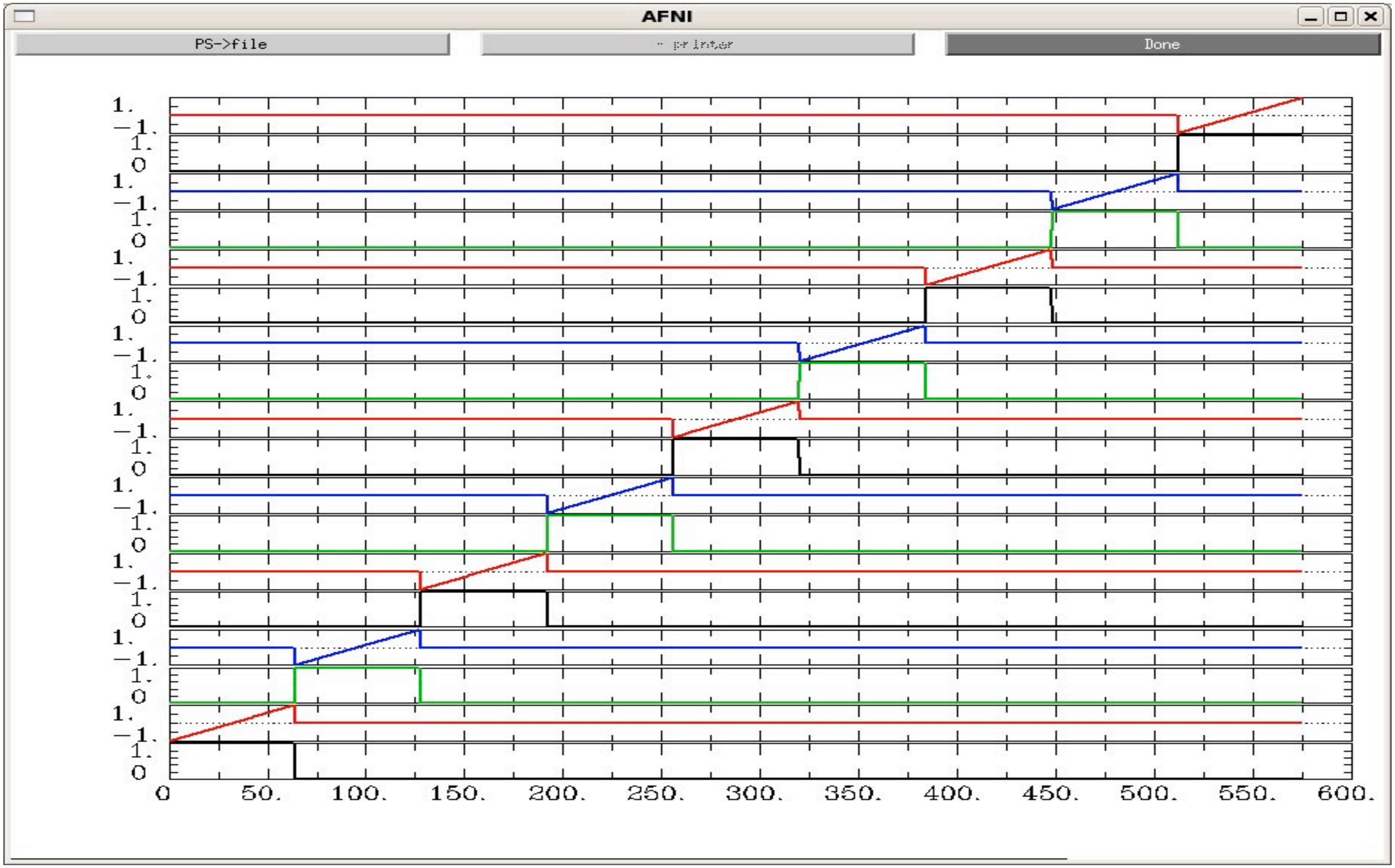
- the 9 visual stimulus classes were given using `-stim_times`
- **it is important to include motion parameters as regressors**
  - this helps to exclude stimulus correlated motion artifacts
  - the 6 motion parameters were given using `-stim_file`
  - `3dvolreg` has previously been run, with the `-1Dfile` option

Regressor Matrix for This Script (via -xjpeg)



- 18 baseline regressors
  - linear baseline
  - 9 runs times 2 params
- 9 visual stimulus regressors
  - 3x3 stimulus design
- 6 motion regressors
  - 3 shifts, 3 rotations

Regressor Matrix for This Script (via -x1D)

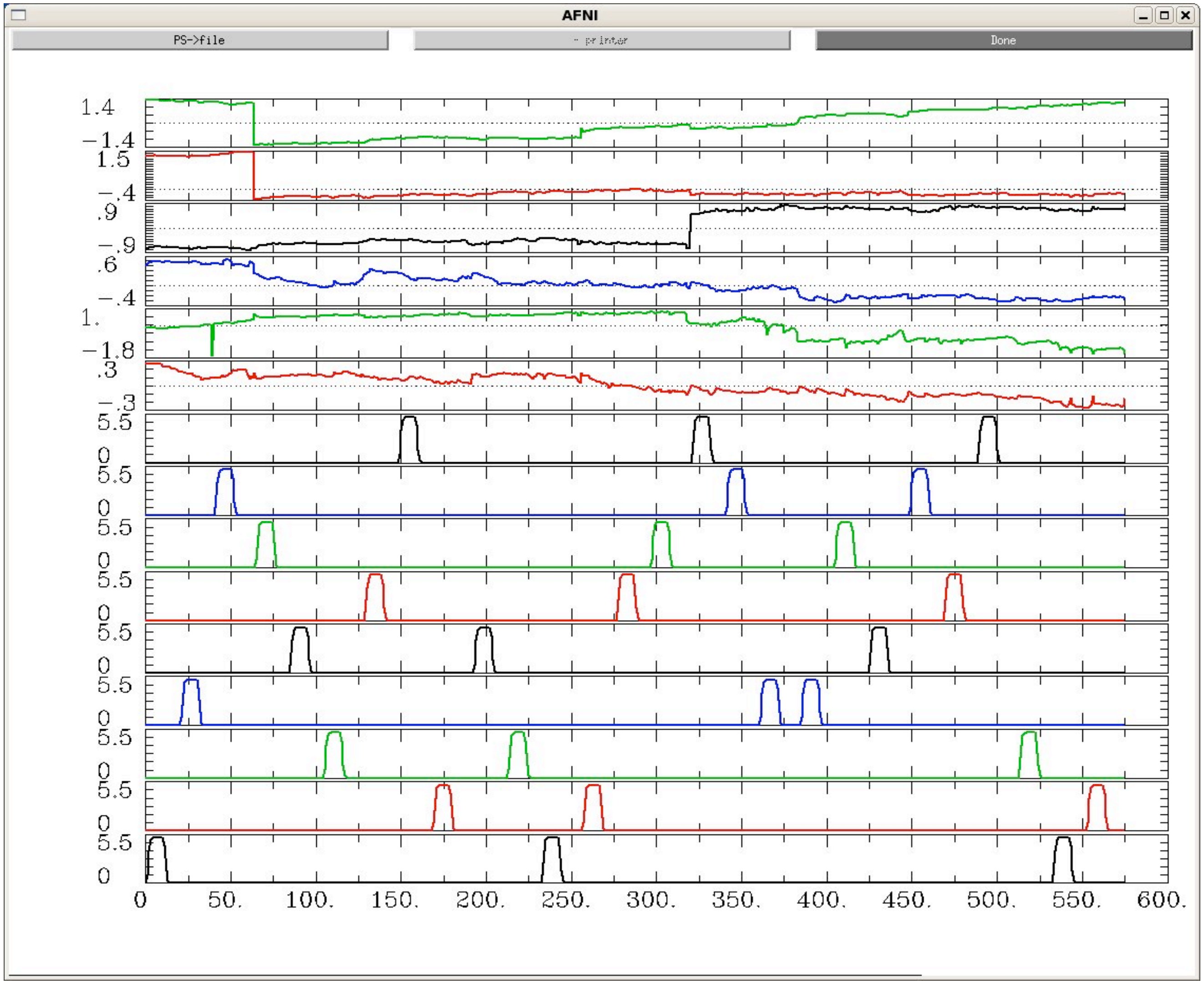


baseline regressors: via **1dplot -sepscl xmat_rall.x1D'[0..18]'**

Regressor Matrix for This Script (via -x1D)

• motion regressors

• visual stimuli



1dplot -sepscl xmat_rall.x1D'[18..$]'

Novel Features of 3dDeconvolve - 1

```
-concat '1D: 0 64 128 192 256 320 384 448 512'
```

- "File" that indicates where distinct imaging runs start inside the input file
  - Numbers are the time indexes inside the file for start of runs
  - In this case, a .1D file put directly on the command line
    - Could also be a filename, if you want to store that data externally

```
-num_stimts 15
```

- We have 9 visual stimuli (+6 motion), so will need 9 **-stim_times** below

```
-stim_times 1
 '1D: 0.0 * | | | 120.0 | | | | | 60.0'
 'BLOCK(20,1)'
```

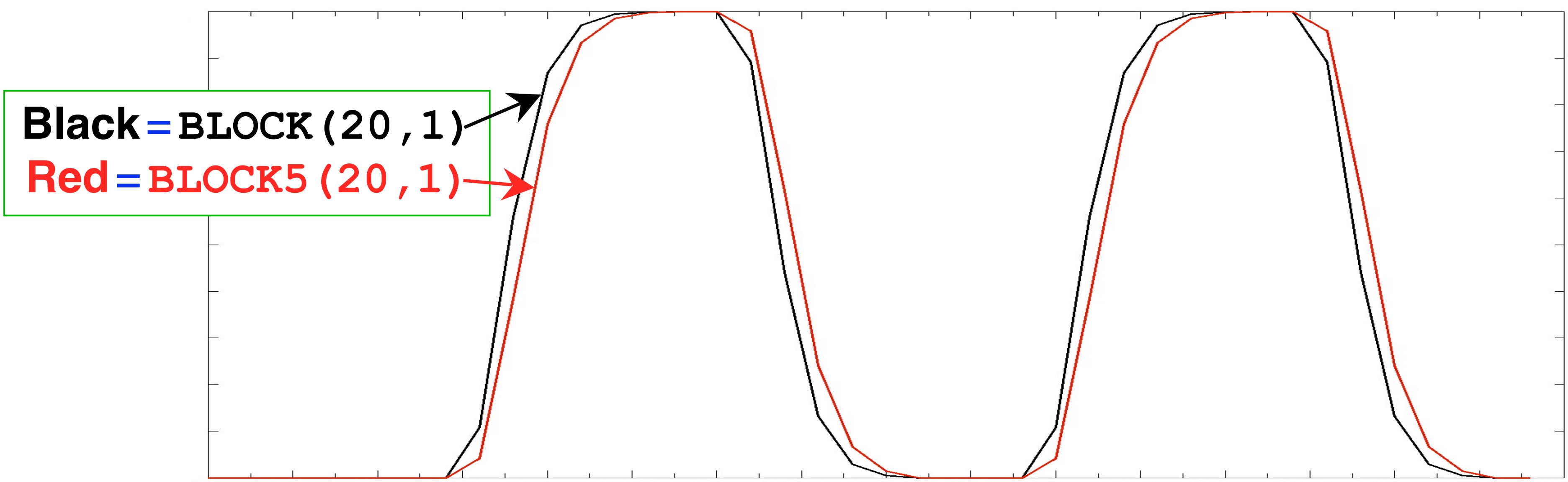
- "File" with 9 lines, each line specifying the start time in seconds for the stimuli within the corresponding imaging run, with the time measured relative to the start of the imaging run itself
- HRF for each block stimulus is now specified to go to maximum value of 1 (compare to graphs on previous slide)
  - This feature is useful when converting FMRI response magnitude to be in units of percent of the mean

Aside: the `'BLOCK()'` HRF Model

- `BLOCK(L)` is convolution of square wave of duration `L` with "gamma variate function" $t^4 e^{-t} / [4^4 e^{-4}]$ (peak value=1 at t=4):

$$h(t) = \int_0^{\min(t,L)} s^4 e^{-s} / [4^4 e^{-4}] ds$$

- "Hidden" option: `BLOCK5` replaces "4" with "5" in the above
  - Slightly more delayed rise and fall times
- `BLOCK(L,1)` makes peak amplitude of *block* response = 1



Novel Features of 3dDeconvolve - 2

```
-gltsym 'SYM: tpos -epos'  -glt_label 1 TPvsEP
```

- **GLT**s are **G**eneral **L**inear **T**ests
- **3dDeconvolve** provides test statistics for each regressor and stimulus class separately, but if you want to test combinations or contrasts of the β weights in each voxel, you need the `-gltsym` option
- Example above tests the difference between the β weights for the `Positive Telephone` and the `Positive Email` responses
  - Starting with `SYM:` means symbolic input is on command line
    - Otherwise inputs will be read from a file
  - Symbolic names for each stimulus class are taken from `-stim_label` options
  - Stimulus label can be preceded by `+` or `-` to indicate sign to use in combination of β weights
- Goal is to test a linear combination of the β weights
  - Tests if $\beta_{tpos} - \beta_{epos} = 0$
  - e.g., does `tpos` get a bigger response than `epos` ?
- Quiz: what would `'SYM: tpos epos'` test?

It would test if $\beta_{tpos} + \beta_{epos} = 0$

Novel Features of 3dDeconvolve - 3

```
-gltsym 'SYM: tpos tneu tneg -epos -eneu -eneg'
-glt_label 3 TvsE
```

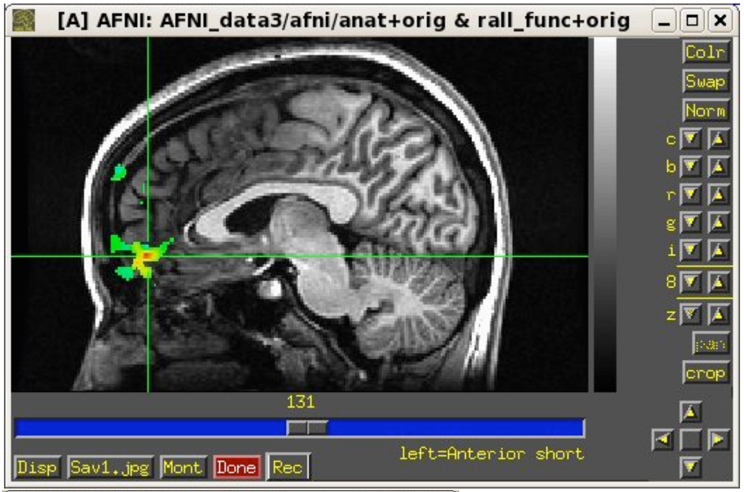
- Goal is to test if $(\beta_{tpos} + \beta_{tneu} + \beta_{tneg}) - (\beta_{epos} + \beta_{eneu} + \beta_{eneg}) = 0$
  - Regions where this statistic is significant have different amounts of (average) BOLD signal change in the telephone tasks versus the email tasks
- `-glt_label 3 TvsE` option is used to attach a meaningful label to the resulting statistics sub-bricks
  - Output includes the ordered summation of the β weights and the associated statistical parameters (t- and/or F-statistics)

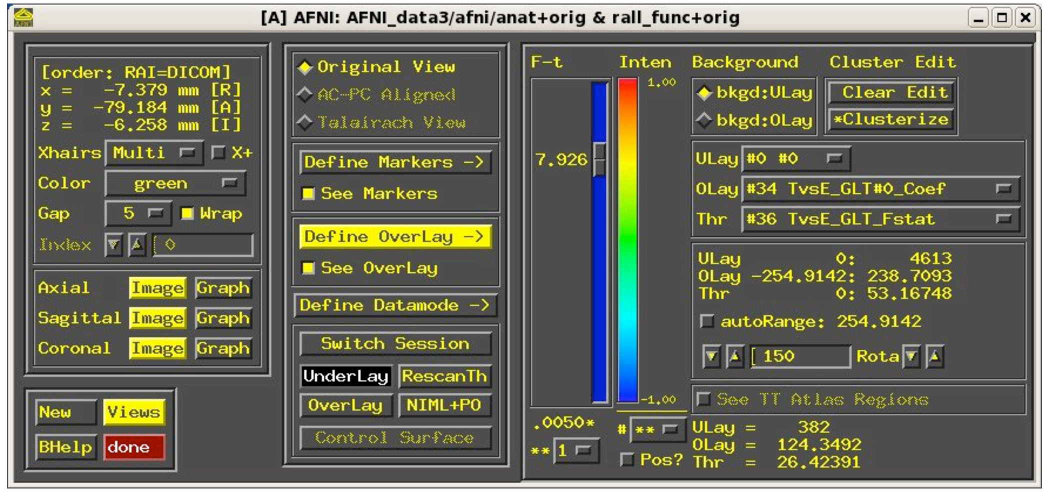
Novel Features of 3dDeconvolve - 4

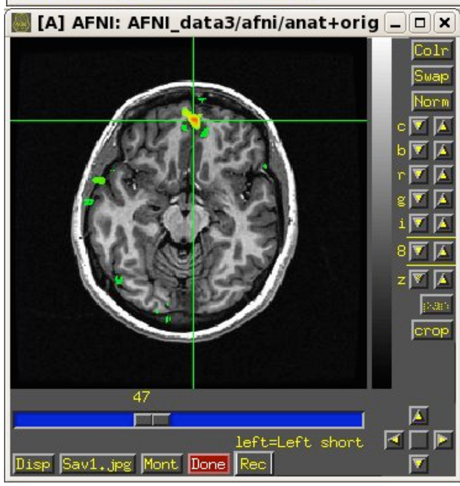
`-fout -tout` = output both F- and t-statistics for each stimulus class (`-fout`) and stimulus coefficient (`-tout`) — but not for the baseline coefficients (if you want baseline statistics: `-bout`)

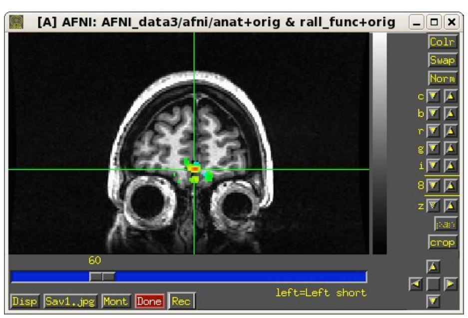
- The full model statistic is an F-statistic that shows how well the sum of all 9 input model time series fits voxel time series data
  - Compared to how well *just* the baseline model time series fit the data times (in this example, have 24 baseline regressor columns in the matrix — 18 for the linear baseline, plus 6 for motion regressors)
- The individual stimulus classes also will get individual F- and/or t-statistics indicating the significance of their individual *incremental* contributions to the data time series fit
  - e.g., F_{tpos} tells if the full model explains more of the data variability than the model with `tpos` omitted and all other model components included

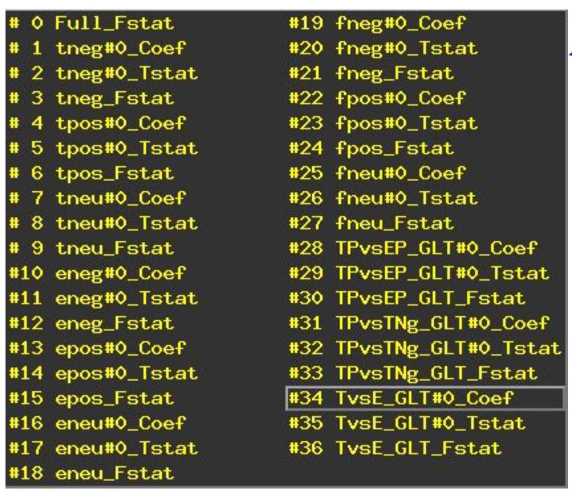
Results of **rall_regress** Script











# 0 Full_Fstat	#19 fneg#0_Coef
# 1 tneg#0_Coef	#20 fneg#0_Tstat
# 2 tneg#0_Tstat	#21 fneg_Fstat
# 3 tneg_Fstat	#22 fpos#0_Coef
# 4 tpos#0_Coef	#23 fpos#0_Tstat
# 5 tpos#0_Tstat	#24 fpos_Fstat
# 6 tpos_Fstat	#25 fneu#0_Coef
# 7 tneu#0_Coef	#26 fneu#0_Tstat
# 8 tneu#0_Tstat	#27 fneu_Fstat
# 9 tneu_Fstat	#28 TPvsEP_GLT#0_Coef
#10 eneg#0_Coef	#29 TPvsEP_GLT#0_Tstat
#11 eneg#0_Tstat	#30 TPvsEP_GLT_Fstat
#12 eneg_Fstat	#31 TPvsTNg_GLT#0_Coef
#13 epos#0_Coef	#32 TPvsTNg_GLT#0_Tstat
#14 epos#0_Tstat	#33 TPvsTNg_GLT_Fstat
#15 epos_Fstat	#34 TvsE_GLT#0_Coef
#16 eneu#0_Coef	#35 TvsE_GLT#0_Tstat
#17 eneu#0_Tstat	#36 TvsE_GLT_Fstat
#18 eneu_Fstat	

- Images showing results from third GLT contrast: **ATvsHL**
- Menu showing labels from **3dDeconvolve** run
- Play with these results yourself*!*

Statistics from 3dDeconvolve

- An *F*-statistic measures significance of how much a model component (stimulus class) reduced the variance (sum of squares) of data time series residual
  - After all the other model components were given their chance to reduce the variance
  - **Residuals** ≡ data – model fit = errors = `-errts`
  - A *t*-statistic sub-brick measures impact of one coefficient (of course, **`BLOCK`** has only one coefficient)
- Full *F* measures how much the all signal regressors combined reduced the variance over just the baseline regressors (**sub-brick #0**)
- Individual partial-model *F*s measures how much each individual signal regressor reduced data variance over the full model with that regressor excluded (e.g., **sub-bricks #3, #6, #9**)
- The **Coef** sub-bricks are the β weights (e.g., **#1, #4, #7, #10**) for the individual regressors
- Also present: GLT coefficients and statistics

# 0 Full_Fstat	#19 fneg#0_Coef
# 1 tneg#0_Coef	#20 fneg#0_Tstat
# 2 tneg#0_Tstat	#21 fneg_Fstat
# 3 tneg_Fstat	#22 fpos#0_Coef
# 4 tpos#0_Coef	#23 fpos#0_Tstat
# 5 tpos#0_Tstat	#24 fpos_Fstat
# 6 tpos_Fstat	#25 fneu#0_Coef
# 7 tneu#0_Coef	#26 fneu#0_Tstat
# 8 tneu#0_Tstat	#27 fneu_Fstat
# 9 tneu_Fstat	#28 TPvsEP_GLT#0_Coef
#10 eneg#0_Coef	#29 TPvsEP_GLT#0_Tstat
#11 eneg#0_Tstat	#30 TPvsEP_GLT_Fstat
#12 eneg_Fstat	#31 TPvsTNg_GLT#0_Coef
#13 epos#0_Coef	#32 TPvsTNg_GLT#0_Tstat
#14 epos#0_Tstat	#33 TPvsTNg_GLT_Fstat
#15 epos_Fstat	#34 TvsE_GLT#0_Coef
#16 eneu#0_Coef	#35 TvsE_GLT#0_Tstat
#17 eneu#0_Tstat	#36 TvsE_GLT_Fstat
#18 eneu_Fstat	

Group Analysis: will be carried out on β or `GLT` coefs from single-subject analyses